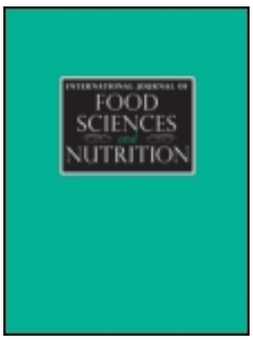

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Microbiological evaluation of ghanaian maize dough co-fermented with cowpea

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Fermented maize dough meals form a large proportion of people's diet in Ghana. To enhance the nutritive value of these carbohydrate-rich foods, protein complementation was introduced. In this study, microbial ecology of fermenting maize dough fortified with 20% cowpea was investigated. A total of 106 microbial strains were isolated from different batches of the fermenting dough at periodic intervals. Ten genera of microorganisms namely *Lactobacillus*, *Leuconostoc*, *Saccharomyces*, *Debaryomyces*, *Candida*, *Bacillus*, *Micrococcus*, *Klebsiella*, *Escherichia* and *Aspergillus* were identified, with lactic acid bacteria species being predominant. A lactic count of log 9.9 cfu/g was obtained at the end of 72 h fermentation relative to log 6.6 cfu/g recorded for aerobic mesophiles. At the 12 h fermentation period, the population of yeasts was less than log 1.0 cfu/g, but gradually rose to log 5.36 cfu/g by 48 h followed a slight decline at the end of 72 h fermentation period (log 4.08 cfu/g). Enteric microorganisms that were isolated from the raw cowpea were less than log 1.0 cfu/g at 12 h of fermentation, while the *Aspergillus* species were isolated from the raw maize and the dough subjected to drying treatment. The growth of inoculated enteropathogenic *E. coli* and *S. typhimurium* was inhibited in the cooked, fermented maize–cowpea dough, and at 72 h, they were not within detectable limit. The study concluded that addition of cowpea at 20% level did not affect the natural fermentation characteristics of the maize dough.

Introduction

Fermented maize forms the basis of a variety of foods in Ghana and other West African countries, contributing to a large proportion of the daily carbohydrate intake (Jespersen *et al.*, 1994). Traditionally, maize grains are subjected to uncontrolled and spontaneous fermentation to produce foods and beverages such as ogi and agidi (infant weaning and adult foods), sekete, akadamu (alcoholic beverages), kenkey and massa (fermented maize dough) (Akinrele, 1970; Odunfa & Adeyele, 1985; Sefa-Dedeh & Asante, 1988; Sanni, 1989; Sefa-Dedeh & Mensah, 1989; Oyeyiola, 1990; Halm *et al.*, 1993).

Since maize is the basic raw material, such products are rich in carbohydrates, relatively low in protein and deficient in some essential amino acids, particularly lysine (Nche *et al.*,

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1994). Due to the limited supply of animal protein, attempts were made by some workers to improve protein quality and quantity of traditional maize-based fermented products in the sub-region by supplementation with cowpea and soybean (Nche *et al.*, 1994; Sanni *et al.*, 1999). However, soybeans are relatively expensive and not well accepted in the sub-region.

On the other hand, cowpea is the most widely consumed legume in West Africa because of its taste and ease with which it is prepared and incorporated into recipes. The beans serve as the largest single contributor to the total protein intake of many rural and urban families in those countries (Onigbinde and Akinyele, 1983). Toasted cowpea was the major ingredient used for the fortification of traditional fermented maize porridge ('pap for health') used for a pilot intervention study in 12 communities in Kwara State, Nigeria (Guptill *et al.*, 1993). Similarly, a low-cost protein energy fermented weaning food of acceptable quality was also prepared using maize, cowpea and crayfish as the major ingredients (Abbey *et al.*, 1988). Nti and Plahar (1995) also reported the chemical and biological characteristics of a maize-based West African traditional weaning food, supplemented with 30% pre-cooked cowpea. However, there is no corresponding information on the microbial profile of these protein-complemented maize products.

In this article, the influence of cowpea fortification on the microbiological status and antibacterial activity of Ghanaian traditional fermented maize dough is presented.

Materials and methods

Preparation of fortified maize dough

Samples (5 each) were randomly collected at periodic intervals from the fermenting batches of the fortified dough in the Food Laboratory of the Department of Nutrition and Food Science, University of Ghana, Accra, Ghana. A batch is defined here as total dough meant for one field trip. The fortified traditional fermented maize dough was used for pilot nutritional intervention study in some selected communities in Ghana. The procedures for the preparation are as follows. Maize grains are sorted out to remove the extraneous matter, washed twice with tap water and steeped in water for 24 h at room temperature (30 ± 1°C). The water is drained off and dehulled cowpea is mixed with the steeped grain at ratio 20:80 and milled. The milled maize–cowpea mixture is molded into doughs by adding water in ratio 1:3 ($^{v}/_{w}$), and the doughs are packed in jute bags in a container and covered tightly. Natural fermentation is allowed to take place for 72 h. The control was the maize dough prepared without the addition of cowpea. For the purpose of this study, samples of the fermented dough were collected and subjected to solar and open-air drying for 4 days. Solar drying was by spreading the fermented dough in a rectangular wooden boxes with polythene sheet as the lid. The boxes were routinely placed in the sun on top of a raised platform. Open-air drying was the traditional method of spreading the dough on a raffia mat or thick polythene sheet placed on a concrete slab.

Microbiological analysis

Isolation procedures. Samples included maize grains, cowpea seeds, fermenting dough, solar and open-dried dough. For all the samples, 10 g were homogenized in 90 ml sterile distilled water using a stomacher (Lab blender Model 80, Seward Medical, London) for 30 sec at 'normal' speed. One ml of appropriate serial dilutions was mixed with molten medium and pour-plated in duplicates. The following media were used: Plate Count Agar (PCA) for total count, de Man Rogosa and Sharpe Agar (MRS, pH 5.5) for lactic acid bacteria, Potato Dextrose Agar (PDA) acidified with tartaric acid for moulds, Malt Extract Agar (MEA) supplemented with streptomycin for yeasts, Nutrient Agar (NA) for other bacteria and Violet Red Bile Dextrose Agar (VRBD) for enterobacteriaceae. Inoculated MRS plates were incubated anaerobically (BBL Gas Pak Anaerobic System, Cockeysville, Maryland, USA). Incubation of PDA plates was at 25°C for 3 days, while other inoculated media plates were incubated at 30°C for 48 h.

Characterization of the isolates. Distinct colonies were enumerated, purified by conventional streaking method and grouped. Pure cultures of the bacterial isolates were Gram-stained, while cell morphology and motility were examined by phase contrast microscopy. The organisms were further categorised on the basis of their catalase

Table 1. Frequency of occurrence of microrganisms isolated during fermentation maize—cowpea dough

Microorganisms	*Replicate samples*				*No. of isolates*	*% total*
	A	*B*	*C*	*D*		
Lactobacillus plantarum	9	12	10	11	42	39.62
Lactobacillus fermentum	6	4	5	4	19	17.92
Leuconostoc mesenteroides	1	1	1	3	6	5.66
Saccharomyces cerevisiae	2	3	2	3	10	9.43
Debaryomyces hansenii	0	1	3	2	6	5.66
Candida tropicalis	2	1	1	1	5	4.71
Bacillus subtilis	1	1	3	2	7	6.60
Micrococcus spp.	1	2	0	1	4	3.77
Klebsiella spp.	0	1	1	0	2	1.88
Escherichia coli	1	0	1	1	3	2.83
Aspergillus niger	1	1	0	0	2	1.88
Total					106	100

Total no. of samples (n = 20).
A, B, C, D = batches of fermented cowpea-maize doughs for four field trips.

reaction and endospore staining. The isolates were phenotypically characterised using appropriate API identification galleries (API Systems, Biomerieux SA France) and other complementary tests were carried when necessary. API 50 CH was used for LAB, API 20 E for enterics, and API for 20 NE for other bacterial groups. The yeast isolates were subjected to various physiological and biochemical tests which included assimilation of carbon and nitrogen compounds, sugar fermentation, growth at 25, 30, 37 and 50°C, urease test, growth in the presence of actidione and gelatin liquefaction. Reference to standard descriptions in *Bergey's Manual of Systematic Bacteriology* (Sneath *et al.*, 1986) and 'The yeasts, a taxonomic study' (Kreger -Van Rij, 1984) was made for confirmation of the phenotypic identification. Moulds were identified according to the procedures outlined by Barnet (1960).

Antibacterial activity of the fermented dough samples

Enteropathogenic *Escherichia coli* NCTC 10418 and *Salmonella typhimurium* ATCC 13311 were obtained from the Culture Collection of Department of Medical Microbiology, College of Medicine, University of Ibadan, Nigeria. The organisms were propagated in Brain Heart Infusion (BHI) broth. For the preparation of porridge, the method of Nout *et al.* (1989) was adopted. The appropriate volume of tap water was brought to a vigorous boil and the corresponding quantity of the dough samples was added while stirring to avoid lumps.

Cooking was allowed to progress for 10 min at 100°C and 300 ml each of the resulting porridge was poured into sterile conical flasks. After cooling to about 45°C, the flasks were singly inoculated (10^7 cfu/ml porridge) with the test organisms. Vigorous mixing was done to ensure even distribution of the inoculum, and incubation of the inoculated flasks was at 30°C for 72 h. Viable count was estimated at periodic intervals on VRBD agar plates.

Results and discussion

A total of 106 microbial strains were isolated from raw maize grains, cowpea seeds, fermenting dough, solar and open-air dried fermented dough. Ten genera of microorganisms, namely *Lactobacillus*, *Leuconostoc*, *Saccharomyces*, *Debaryomyces*, *Candida*, *Bacillus*, *Micrococcus*, *Klebsiella*, *Escherichia* and *Aspergillus* were identified, with lactic acid bacteria recording a total occurrence of 63% (Table 1). The dominance of lactic acid bacteria in traditional fermentation of maize-based products was reported by some workers (Fields *et al.*, 1981; Halm *et al.*, 1993). *L. plantarum*, which had 42% occurrence in this study, is reported to be mainly responsible for the production of lactic.

Table 2. Microbial count of the raw maize grains and the cowpea seeds

Microorganisms (log cfu/g)	Maize	Cowpea
Total count	5.18 ± 1.22	5.54 ± 1.43
Lactic acid bacteria	<1.00 (–)	<1.00 (–)
Enterobacteriaceae	<1.00 (–)	3.30 ± 0.74
Other bacteria species	4.15 ± 1.00	5.70 ± 1.85
Yeasts	<1.00 (–)	<1.00 (–)
Moulds	5.92 ± 0.62	<1.00

Values are average of duplicate determinations ($n = 4$) with standard deviations.
cfu = colony-forming units; <1.00 = less than 1.0 (limit of detection); (–) = not calculated.

Although yeasts were also isolated, their importance during maize dough fermentation is yet to be defined. However, Akinrele (1970), reported that *S. cerevisiae* and *Candida mycoderma* contribute to flavour acceptability of ogi, a traditional Nigerian fermented infant weaning and adult food made from maize.

Table 2 shows the colony-forming units of the raw maize and cowpea. Lactic acid bacteria were not isolated, but the total microbial counts were log 5.54 cfu/g and log 5.18 cfu/g for cowpea and maize respectively. Members of the enterobacteriaceae were only isolated from cowpea seeds (log 3.30 cfu/g), while yeasts were not within detectable limits (<log 1.0 cfu/g). *Aspergillus* species were only isolated on raw maize, solar and open-air dried fermented doughs. The microbial load of the fermenting dough is presented in Table 3. The population of lactic acid bacteria rose from log 2.25 cfu/g at the 0 h of fermentation to log 9.90 cfu/g at the end of 72 h fermentation period. A final value of log 4.6 cfu/g was obtained for the yeast population. However, in contrast to the count of log 3.30 cfu/g for the enteric microorganisms from the raw cowpea seeds, they were not within limit of detection (<log 1.0 cfu/g) in the fermenting dough. The antimicrobial compounds produced by lactic acid bacteria in the fermenting matrix could have inhibited their growth.

Table 3. Microbial count of the fermenting dough

	Fermentation period (h)			
Microorganisms (log cfu/g)	*0*	*24*	*48*	*72*
Total count	4.49^{b} $(4.58)^{b}$	3.66^{c} $(3.70)^{c}$	4.36^{b} $(4.34)^{b}$	6.63^{a} $(6.62)^{a}$
Lactic acid bacteria	2.25^{e} $(2.32)^{e}$	4.53^{c} $(2.51)^{d}$	6.67^{b} $(6.69)^{b}$	9.89^{a} $(9.91)^{a}$
Enterobacteriaceae	2.08^{a} $(<1.00)^{b}$	$(<1.00)^{b}$ $(<1.00)^{b}$	$(<1.00)^{b}$ $(<1.00)^{b}$	$(<1.00)^{b}$ $(<1.00)^{b}$
Other bacteria species	3.48^{d} $(2.51)^{e}$	3.61^{d} $(3.60)^{d}$	4.41^{c} $(5.08)^{b}$	5.79^{a} $(5.78)^{a}$
Yeasts	3.00^{d} $(3.15)^{d}$	2.41^{c} $(2.56)^{c}$	5.36^{a} $(5.30)^{a}$	4.00^{b} $(4.08)^{b}$
Moulds	$(<1.00)^{a}$ $(<1.00)^{a}$	$(<1.00)^{a}$ $(<1.00)^{a}$	$(<1.00)^{a}$ $(<1.00)^{a}$	$(<1.00)^{a}$ $(<1.00)^{a}$

Values represent the mean scores ($n = 20$). Sample means having the same letters for each microbial group are not significantly different ($P > 0.05$).
Figures in parentheses are the values for the fermenting dough without the addition of cowpea. cfu = colony-forming units; <1.00 = less than 1.0 (limit of detection).

Table 4. Microbial count of solar and open-air dried fermented maize dough

	Drying period (days)							
	1		*2*		*3*		*4*	
Microorganisms (log cfu/g)	*SD*	*OD*	*SD*	*OD*	*SD*	*OD*	*SD*	*OD*
Total count	4.91^{b} $(4.88)^{b}$	4.95^{b} $(4.92)^{b}$	4.81^{b} $(4.79)^{b}$	4.98^{b} $(4.94)^{b}$	3.62^{c} $(3.65)^{c}$	3.79^{c} $(3.62)^{c}$	2.60^{d} $(2.81)^{d}$	6.89^{a} $(3.62)^{c}$
Lactic acid bacteria	7.67^{a} $(7.70)^{a}$	5.75^{b} $(5.73)^{b}$	5.36^{b} $(5.41)^{b}$	4.23^{c} $(4.30)^{c}$	3.54^{d} $(3.61)^{d}$	2.20^{e} $(2.23)^{e}$	2.34^{e} $(3.36)^{d}$	2.20^{e} $(2.26)^{e}$
Enterobacteriaceae	$<1.0^{b}$ $(<1.0)^{b}$	$<1.0^{b}$ $(<1.0)^{b}$	$<1.0^{b}$ $(<1.0)^{b}$	2.00^{a} $(2.08)^{a}$	$<1.0^{b}$ $(<1.0)^{b}$	2.15^{a} $(2.23)^{a}$	$<1.0^{b}$ $(<1.0)^{b}$	2.23^{a} $(2.15)^{a}$
Other bacteria species	4.87^{b} $(4.85)^{b}$	4.80^{b} $(4.81)^{b}$	4.78^{b} $(4.79)^{b}$	4.86^{b} $(4.83)^{b}$	3.26^{c} $(3.15)^{c}$	6.81^{a} $(6.78)^{a}$	2.08^{d} $(3.18)^{c}$	6.87^{a} $(6.88)^{a}$
Yeasts	3.32^{a} $(3.38)^{a}$	3.26^{a} $(3.32)^{a}$	2.23^{b} $(2.20)^{b}$	2.00^{c} $(2.08)^{c}$	$<1.0^{d}$ $(<1.0)^{d}$	$<1.0^{d}$ $(<1.0)^{d}$	$<1.0^{d}$ $(<1.0)^{d}$	$<1.0^{d}$ $(<1.0)^{d}$
Moulds	2.04^{g} $(2.11)^{f}$	2.08^{g} $(2.18)^{f}$	2.00^{g} $(<1.0)^{b}$	3.61^{d} $(3.45)^{d}$	2.15^{f} $(2.11)^{f}$	5.71^{b} $(5.15)^{c}$	2.49^{e} $(2.43)^{e}$	5.73^{b} $(6.23)^{a}$

Values represent the mean scores (n = 20). Sample means having the same letters for each microbial group are not significantly different ($P > 0.05$).
Figures in parentheses are the values for fermented maize dough without the addition of cowpea.
SD = solar drying method; OD = open-air drying method; cfu = colony-forming units; <1.00 = less than 1.0 (limit of detection).

Table 4 shows the microbial load of the dough samples subjected to solar and and open-air drying. A decrease in the lactic acid bacteria population was obtained in the dough samples from both drying methods, while the total microbial count increased till the end of the 4 day drying treatment. The open-air dried dough had an enteric count of log 2.15 cfu/g, which may have been due to contamination from the open environment.

Table 5. Growth kinetics (log cfu/g) of *E. coli* NCTC 10418[1] and *S. typhimurium* ATCC 13311[2] inoculated into cooked dough samples

	Dough samples			
Incubation period (h)	*UFD*	*FFD*	*SDFD*	*ODFD*
0	[1]7.51^{a} [2]7.54^{a}	7.48^{b} 7.48^{b}	7.43^{b} 7.48^{b}	7.53^{a} 7.46^{b}
24	8.08^{b} 8.41^{a}	4.08^{f} 3.91^{g}	4.88^{c} 4.57^{d}	4.91^{c} 4.38^{e}
48	8.05^{a} 7.71^{b}	1.36^{e} 1.26^{f}	1.40^{d} 1.30^{e}	1.52^{c} 1.31^{e}
72	7.83^{a} 7.23^{b}	$<1.00^{c}$ $<1.00^{c}$	$<1.00^{c}$ $<1.00^{c}$	$<1.00^{c}$ $<1.00^{c}$

Values represent mean scores (n = 4).
UFD = unfermented maize—cowpea dough; FFD = fermented maize-cowpea dough; SDFD = solar-dried fermented maize—cowpea dough; ODFD = open-air dried fermented maize—cowpea dough; cfu = colony-forming units; <1.00 = less than 1.0 (limit of detection).

The viability of enteropathogenic *E. coli* and *S. typhimurium* in the cooked, fermented and unfermented dough is shown in Tables 5 and 6. There was a sharp decline in the growth of bacterial strains in the fermented cooked dough samples over a period of time, and they were not detected by the 72 h when the experiment was terminated. On the other hand, the unfermented cooked dough samples recorded gradual increase in the growth of the inoculated enteric strains followed by a slight decline. However, at 72 h, the viable count of both strains was above log 7.0 cfu/g. Mensah *et al.* (1988) obtained a similar reduction in the population of four strains *of Shigella flexneri* inoculated into unfortified fermented Ghanaian maize dough. Nout *et al.* (1989) also reported the death of *S. typhimurium* and *Staphylococcus aureus* inoculated into cereal porridges having a pH of less than 4.0. Mensah *et al.* (1991) further confirmed the inhibitory effect of unfortified fermented Ghanaian maize dough against other species of enteric pathogens. Svanberg *et al.* (1992) observed that the growth of enterotoxigenic *E. coli*, *Campylobacter jejeuni*, *Shigella flexneri* and *Salmonella typhimurium* was strongly inhibited in a fermented sorghum with a pH of less than 4.0.

From this investigation, solar and open-air drying methods did not have any negative effect on the antimicrobial activity of the fermented dough. Nout *et al.* (1989) observed that cooked fermented porridge subjected to drum-drying and reconstituted still exhibited inhibitory activity against the inoculated enteric pathogens of the study. According to them, losses of organic acids due to evaporation during drum-drying are negligible. In conclusion, therefore, addition of cowpea at 20% level did not affect the natural fermentation characteristics of the maize nor its antibacterial activity.

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